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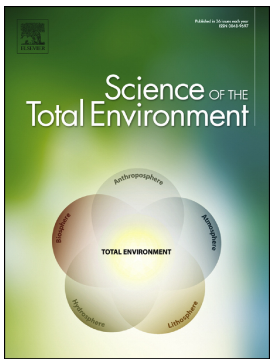

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LC-HRMS Suspect Screening to Show Spatial Patterns of New Psychoactive Substances Use in Australia

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Abstract

New Psychoactive Substances (NPS) are an ever-changing class of compounds designed to imitate the effects of current recreational drugs. Such a diverse market is difficult to assess by traditional means, while collected information can become obsolete before it is available. Wastewater-based epidemiology is one technique which can capture information on where and when NPS appear at the community level. The aim of this study was to identify NPS in wastewater samples using a suspect screening approach. Weekend samples were collected from 50 wastewater treatment plants from Australian capital cities and regional areas across all eight States and Territories and screened against a database containing almost 200 NPS. A total of 22 different NPS were found across all regional and metropolitan wastewater treatment plants. Results showed that the most detected compounds were of the cathinone class, with both Alpha-PVP and methcathinone found in every region. In addition, five different synthetic cannabinoids were detected, at least once in half of the regions analysed. Herein, we report the first comprehensive nationwide analysis of NPS and show the utility of liquid chromatography-high resolution mass spectrometry screening for delivering spatial information of the NPS being consumed in communities.

Keywords: Wastewater, Synthetic Cathinones, High Resolution Mass Spectrometry, Liquid Chromatography, Cannabinoids

Introduction

New psychoactive substances (NPS) are compounds designed to mimic the effects of existing recreational drugs. The most common NPS are synthetic cannabinoids and synthetic cathinones, together accounting for almost 80% of all NPS seizures in Europe and 40% in Australia. (Australian Criminal Intelligence Commission, 2015; EMCDDA, 2018) Legislation has been introduced in many countries to address the NPS market, including specific NPS-related legislation. (UNODC - United Nations Office on Drugs and Crime, 2017)

Current means to monitor NPS use and exposure such as surveys, seizure data and forensic identification have known limitations. Surveys are of limited value as users may often be unaware of or ill-informed as to the compounds present in the substances they are using and there is an inherent self-reporting bias for questions concerning illegal activities. Seizures can be of value, but their sporadic nature means that they cannot provide a profile of NPS use in a community at any one time. Forensic identification of substances being used by people who have died, experienced severe overdoses requiring hospitalisation or otherwise come to attention of police continues to be of importance, but is biased towards drugs such as opioids that produce life-threatening consequences. As an alternative, wastewater analysis has emerged as a means to profile NPS use, as it provides objective identification of the compounds used across the range of different substances. In addition, through repeated testing that generates near real-time information, wastewater analysis can demonstrate trends in NPS use over both short and long term periods.

The analysis of NPS in wastewater has been based on targeted, quantitative liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods, focussing on a limited number of analytes. (Bade et al., 2017; Borova et al., 2015; González-Mariño et al., 2016;

Kinyua et al., 2015; Reid et al., 2014b; Senta et al., 2015) With ever-expanding numbers of NPS, targeted methods based on sequential detection become self-limiting. Furthermore, reference standards and internal standards are required for these methods which are not always commercially available. In cases such as NPS, the most important consideration may not necessarily be to quantify substances with risk potential, but rather demonstrating that they are consumed in a population and where detections take place.

To circumvent these limitations, the qualitative screening analysis of wastewater is an emerging alternative, using LC-high resolution mass spectrometry (HRMS). Hybrid HRMS instruments such as quadrupole-time of flight (QTOF) and linear ion trap-Orbitrap (LTQ-Orbitrap) allow the acquisition of accurate-mass full-spectrum data. (Kaufmann et al., 2010) Compounds suspected to be present (i.e. "suspects") can be extracted from the full-spectrum data. (Asghar et al., 2018; Park et al., 2018; Singer et al., 2016) These data allow one to screen for compounds in a "suspect" or "post-target" way without the need to pre-select the analytes for method development as in quantitative targeted methods. (Hernández et al., 2016; Krauss et al., 2010) In principle, the number of compounds able to be investigated is primarily limited by the sample preparation method, with samples screened against databases containing the accurate mass of the parent compound, fragment ions and retention time (if known).

Few qualitative studies have focussed solely on NPS in wastewater, with less than six NPS found in any one study. (Baz-Lomba et al., 2016; Causanilles et al., 2017; González-Mariño et al., 2016; Reid et al., 2014a) All of these studies were performed in Europe, with some targeting specific events, such as a festival to give a greater chance of capturing NPS use among young people. (Causanilles et al., 2017) The current work presents the qualitative NPS screening of wastewater from 50 sites which captured almost 60 % of the total population across metropolitan and regional Australia. Weekend samples were screened against a

database of more than 200 NPS, using LC-QTOF-HRMS to identify the compounds used on a national level. Spatial differences associated with regional and metropolitan populations are also presented.

2. Materials and Methods

2.1 Chemicals and Reagents

A total of 85 NPS reference standards in the form of mixed standard solutions in methanol were made available for use by Forensic Science South Australia (FSSA) for the screening method. The mixed solutions were supplied in accordance with the appropriate licencing conditions at both the FSSA and the University of South Australia sites. In addition, 4-fluoroamphetamine (4-FA), 4-fluoromethcathinone (4-FMC), N-(Adamantan-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide (5F-APINACA), 4-methylethcathinone (4-MEC), N-[(2S)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)indazole-3-carboxamide (AB-CHMINACA), N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA), 3,4-dichloro-N-{[1-(dimethylamino)cyclohexyl]methyl}benzamide (AH-7921), 1-(5-fluoropentyl)-3-(naphthalen-1-oyl)indole (AM-2201), N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), butylone, butyryl fentanyl, ethylone, furanyl fentanyl, methiopropamine, methoxamine, pentedrone, pentylone, 3,4-Dichloro-*N*-[(1*R*,2*R*)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide (U-47700), (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone (UR-144) and valeryl fentanyl were purchased from Novachem (Collingwood, Victoria, Australia) in concentrations of 100 µg/mL or 1 mg/mL. A mixed stock solution of these latter NPS was prepared at a concentration of 20 ng/L for the screening method in addition to the aforementioned FSSA mixed standards.

Glacial acetic acid, sodium acetate, isopropanol, ammonia (28 %) and formic acid (99 %) were purchased from VWR Chemicals (Tingalpa, Queensland, Australia), while methanol, hydrochloric acid (37 %) and dichloromethane were purchased from Merck (Kilsyth, VIC,

Australia), and sodium metabisulfate ($Na_2S_2O_5$) from Chem-Supply (Gillman, SA, Australia) Ultrapure water was prepared using an Arium® pro VF system (Sartorius Stedim biotech).

2.2 Samples

Influent wastewater (IWW) samples from fifty regional and metropolitan sites around Australia were collected in August 2016 to cover the representative population density across the country. (Australian Criminal Intelligence Commission, 2017) Australia is divided into eight States and Territories: Australian Capital Territory (ACT), New South Wales (NSW), Northern Territory (NT), Queensland (QLD), South Australia (SA), Tasmania (TAS), Victoria (VIC) and Western Australia (WA) with the majority of the population living in metropolitan areas near the coast and predominately on the Eastern seaboard. All of these States and Territories are shown in **Figure 4**. In this work, a metropolitan catchment area was regarded as greater than 100,000 inhabitants. Although at least one capital city site per State/Territory was analysed, some were small enough to fall below our threshold for metropolitan. Nevertheless, they were regarded as metropolitan. Capital city sites are noted with a "*" in **Table S1**.

Details pertaining to number of samples collected, sampling mode and frequency of sampling are provided in **Table S1**. For this work, two samples were analysed for each site, corresponding to the weekend (Saturday and Sunday). A few sites were unable to sample during the weekend, so two weekday samples were analysed instead.

Samples were collected onsite using refrigerated (4 °C) autosamplers at each wastewater treatment plant. Sampling mode and frequency were optimised to provide the most representative samples based on the equipment available as outlined by Ort *et al.* (Ort et al., 2010). Samples were aliquotted into 500 mL HDPE bottles, preserved with final concentration 2 g/L sodium metabisulfite and stored at -20 °C prior to extraction and analysis

2.3 Sample Pre-treatment

Sample pre-treatment including solid phase extraction (SPE) were performed as outlined in our previous work (Bade et al., 2018). Briefly, samples were warmed to room temperature then filtered under vacuum using glass microfibre filters GF/A 1.6 µm (Whatman, Kent, U.K.). 10% Acetic acid was added to lower the pH (4.5-5) of the samples. The acidified samples were loaded onto mixed-mode SPE cartridges (UCT XRDAH (UCT Inc., Bristol, PA, USA); 500 mg/6 mL) which had been conditioned with methanol (6 mL) and sodium acetate buffer (20 mM pH 5, 6 mL). These cartridges incorporate a reverse phase (C8) and Ion Exchange (benzenesulfonic acid) sorbent to allow extraction of a broad range of compounds. The cartridges were successively washed with sodium acetate buffer (6 mL), 0.1 M acetic acid (2 mL) and methanol (6 mL). Analytes were eluted with a mixture of dichloromethane:isopropanol:ammonia (80:16:4, 6 mL) and evaporated to 200 µL under nitrogen at 40°C, when 1% HCl in methanol was added, then evaporated to dryness. The dry residue was reconstituted with 0.1% formic acid in methanol (20 µL) and 0.1% formic acid in MilliQ water (180 µL). Analyses were performed by injecting 10 µL in the LC-QTOF-MS.

2.4 Instrumentation

LC–MS analyses were conducted using a Shimadzu UHPLC pump system (LC-30AD, Kyoto, Japan), Shimadzu autosampler (SIL-30AC), degasser (DGU-20A5), Shimadzu Column Oven (CTO-20AC) and a Valco diverter valve coupled to a Sciex Triple TOF 5600 time-of-flight mass spectrometer (Sciex, Toronto, Canada), fitted with an electrospray (ESI) interface and operated in positive ion mode.

The chromatographic separation was carried out using a Phenomenex Kinetex PFP column (100 x 2.1 mm) with an internal diameter of 1.7 µm connected to a PFP(2) guard column

(SecurityGuard; 4 x 2.0 mm; Phenomenex Inc., Torrance, CA, USA), at a flow rate of 0.3 mL min^{-1} and column oven temperature of 40 °C. The mobile phases used were water with 5% methanol and 0.1% formic acid (solvent A) and methanol with 5% water and 0.1% formic acid (solvent B). The initial percentage of B was 5% and after 2 min was linearly increased to 100% over 11 min, followed by a 4 min isocratic period, then returned to initial conditions in 0.1 min and remained steady for the final 0.9 min. The total run time was 18 min.

MS data were collected over an *m/z* range from 50 – 600. Data were acquired in Sequential Window Acquisition of all THeoretical fragment-ion spectra (SWATH) mode, with all specific information in Bade *et al.* (Bade et al., 2018). Briefly, one TOF MS full scan at low collision energy (CE) of 10 V gave information pertaining to the $[M+H]^+$ and 33 SWATH experiments from *m/z* 50-600 with a 16.2 Da offset and 1 Da overlap (i.e. experiment 2 was between *m/z* 50 – 66.2, experiment 3 between *m/z* 65.2 –82.4, experiment 4 between *m/z* 81.4 – 98.6 etc.) gave information on the fragment ions. Each of these SWATH experiments had CE ranging from 12.0 to 42.7 V depending on the mass range. Experiment 2 had a CE of 12.0 V, experiment 3 of 12.9 and each additional experiment had a CE 0.93 V higher, up to experiment 34 with a CE of 42.7 V. All experiments had a declustering potential of 60 V, while all SWATH experiments had a collision energy spread of 15 V, ion release delay of 67 ns and ion release width of 25 ns. Mass calibration was performed prior to each batch run to ensure mass accuracy using the Sciex ESI Positive Calibration Solution.

Data were acquired in positive mode as all of the compounds under investigation could be analysed in positive mode using Analyst and processed using MultiQuant 2.1.1 and PeakView 2.2.

2.5 Criteria for Qualitative Screening Analysis

The processing workflow for this work was the same as that previously published. (Bade et al., 2018). The retention times were attained for each of the standard compounds with MultiQuant 2.1.1, using the exact mass of the $[M+H]^+$ ± 2 mDa and searching for the largest peak. All entries were then manually checked to ensure that the correct peak (and retention time) had been assigned. Any compounds for which no information was available were manually searched using PeakView and the appropriate SWATH experiment to find fragment ions.

Compounds were detected using one accurate mass ion – commonly the protonated molecule ($[M+H]^+$) at a mass error ± 2 mDa and retention time agreement with a reference standard (± 2%). Confirmation of the identity of the compound detected involved at least two accurate mass ions (± 2 mDa), one of which being the protonated molecule ($[M+H]^+$), and agreement of retention time and isotopic pattern with a reference standard (± 2%). Tentative identifications were made in those cases when the reference standard was not available at the laboratory. It was based on the presence of at least two accurate mass ions (mass error ± 2 mDa), one of which being the protonated molecule ($[M+H]^+$) supported by literature mass data on the suspect compound. Examples of confirmation, detection and tentative identification are given in **Figure 1, 2 and 3**.

2.6 Database for Qualitative Screening Analysis

A database including 105 NPS reference standards and exact mass information of an additional 82 NPS were used in this work. In total, 187 NPS were in the database (**Table S2**). Due to the dearth of known metabolic pathways for most NPS, the majority of the substances in this database were parent drugs rather than metabolites. The selection of NPS reference standards relied on information from FSSA's illicit drug laboratory, Australian Police and forensic laboratories intelligence as well as media and published reports (such as the

EMCDDA early warning system and the Australian National Drug and Alcohol Research Centre bulletin of drugs and the internet (Roxburgh et al., 2017). This was a compromise between exhaustive NPS coverage and finite resources, but, encapsulated a significant number of NPS likely to be encountered in the context of this study. (Partridge et al., 2018)

3. Results and Discussion

3.1 Qualitative Compound Recovery

The SPE method used in this study has been utilised by our group for many studies and is generic enough to allow analysis of a wide range of pharmaceuticals and illicit drugs. (Bade et al., 2018; Tscharke et al., 2016) Nevertheless, an SPE recovery experiment was conducted on a range of NPS (4-FA, 4-FMC, 4-MEC, 5F-APINACA, AB-CHMINACA, AB-PINACA, AH-7921, AM-2201, APINACA, butylone, butyryl fentanyl, furanyl fentanyl, methiopropamine, methoxetamine, pentedrone, pentylone, UR-144 and U-47700). These 18 NPS cover a range of NPS families (cathinones, phenethylamines, cannabinoids and opioids) and with the structural similarities within NPS classes, recovery for these NPS was assumed for all other analogues. Relative recovery was tested based on the work of Matuszewski *et al.* (Matuszewski et al., 2003), where two sets of samples were made. Set 1 consisted of a mixed standard solution (10 ng/L) in solvent, including methylone-d_3 as the only internal standard suitable for these compounds. Set 2 included non-preserved IWW samples from a WWTP in South Australia which were first spiked at the same concentrations as the previous set and then extracted. Both sets were made in triplicate. Relative recovery was calculated using the following equations:

Relative recovery = $(average\ peak\ area\ (Set\ 2))/(average\ peak\ area\ (Set\ 1)\ )$

Corrected relative recovery =

$$\frac{(average\ peak\ area\ (Set\ 2, methylone-d3)/average\ peak\ area\ (Set\ 1, methylone-d3)\ )}{(average\ peak\ area\ (Set\ 2)/average\ peak\ area\ (Set\ 1))}$$

All of the aforementioned substances except for AB-CHMINACA and AB-PINACA were successfully recovered (**Table S3**). As the vast majority of the NPS could be recovered, no separate SPE method was evaluated. It is thus recognised that the absence of these two AB-

substituted cannabinoids from the samples may not be due to their lack of use by the population but rather the sample extraction method.

3.2 Identification of NPS in Wastewater

A total of 22 NPS were found, of which 10 were confirmed (**Table 1**). Examples of compounds that were confirmed, detected and tentatively identified are in **Figure 1** (methoxetamine confirmed), **Figure 2** (5F-APINACA detected), and **Figure 3** (monohydroxylated 5F-APINACA, tentatively identified). Four classes of NPS were found: synthetic cathinones, cannabinoids, opioids, amphetamine analogues as well as methoxetamine and methiopropamine. Overall, NPS were detected in 178 instances, with over half confirmed. The instrumental limit of detection (ILOD) was estimated for all of the NPS found, by analysing standards from 0.1 ng/L to 10 ng/L (**Table S4**). The ILOD was deemed the concentration at which the protonated molecule and at least one fragment ion had a signal/noise ratio above 3.

Synthetic cathinones were the most common NPS class found, with methcathinone and alpha-PVP the most frequently detected and confirmed substances. This is in agreement with Australian seizure data, with synthetic cathinones accounting for one-third of all analysed NPS seizures in 2015-16. (Australian Criminal Intelligence Commission, 2015) All NPS detected and confirmed, together with the sites are in **Table S5**. It is interesting to note that five synthetic cannabinoids were detected, even though they have previously been reported as being extensively metabolised, and generally require specific sample treatment. (González-Mariño et al., 2018; Reid et al., 2014b) The metabolites of these synthetic cannabinoids (i.e. 5F-APINACA, AM-2201, JWH-018, JWH-073 and UR-144) were also included in the suspect database, using literature data. (Holm et al., 2015; Scheidweiler et al., 2015) Two metabolites were tentatively identified (monohydroxylated 5F-APINACA and UR-144 *N*-

pentanoic acid) based on the accurate mass of the protonated molecule and fragment ion(s). However, reference standards are needed to fully confirm their identity.

3.2.1 Spatial Distribution

Australia is made up of eight States and Territories (**Figure 4**). The majority of the population live in metropolitan areas near the coast. NSW is the most populous state (7.7 million inhabitants) and has the most populated city, Sydney (5.37 million inhabitants). WA has by far the largest area but a relatively small population of only 2.6 million, over half of which live in Perth – a coastal city. TAS, ACT and NT have the smallest populations, all of which total less than 520,000.

At least one NPS was detected in every State/Territory (**Table S5** and **Figure 4**). The most commonly found NPS (alpha PVP and methcathinone) were confirmed in at least one site in all States/Territories. Both of these have previously been found in South Australia (Chen et al., 2013; Tscharke et al., 2016) but this work shows that their use is not confined to this one State. MDA was found in more than half of the sites. It is a known metabolite of MDMA, with 1.65% excreted as MDA. (Khan and Nicell, 2011) It is thus possible that the MDA seen in this study is due to MDMA metabolism. But as this work is qualitative, standalone MDA use cannot be ruled out. In contrast, the synthetic cannabinoids AM-2201 and UR-144 were only detected in QLD on the Eastern coast, while 5F-APINACA was detected in QLD and WA. Ethylone was seen in all States/Territories except VIC.

Synthetic cathinones, such as methylone, ethylone, butylone and pentylone, have previously been reported as adulterants in 'ecstasy' pills, while methoxetamine and 4-FA have been found as adulterants of amphetamine and cocaine. (Brunt et al., 2017; Hondebrink et al., 2015) With this study focussing on weekend samples, it increased the likelihood that possible NPS adulterants of 'party drugs' (i.e. ecstasy, cocaine and amphetamine) could be found.

This has previously been postulated for ethylone use in South Australia and this work shows that cathinones are indeed present around Australia. However, it must also be acknowledged that there are NPS users who know exactly what they are taking and may be specifically consuming these synthetic cathinones and methoxetamine deliberately. Methoxetamine, although detected in ACT, NSW, QLD and SA, was only confirmed in VIC samples.

3.2.2 Metropolitan and Regional Trends

There was little difference between the NPS found in metropolitan and regional areas for most of the compounds in the current study (**Figure 4** and **Table S5**). On average, ACT, QLD and SA metropolitan sites had the most NPS confirmed. For regional sites, NT, QLD, SA and VIC averaged about two confirmed NPS findings per site. No NPS were confirmed in the metropolitan sites of VIC. The most common NPS found, methcathinone, showed relatively similar use in regional and metropolitan sites, with it confirmed in 19 metropolitan sites and 20 regional sites. However, the synthetic cathinones methylone, ethylone and butylone were more commonly found in metropolitan sites, giving credence to the hypothesis of their use as 'party drug' adulterants outlined above due to the more abundant city nightlife.

All of these sites were analysed in August 2016 for the common illicit drugs (cocaine, MDMA and methamphetamine) in the Australian Criminal Intelligence Commission (ACIC) Wastewater Report. (Australian Criminal Intelligence Commission, 2017) In this report, metropolitan ACT and NSW sites were shown to have the highest usage of cocaine, while metropolitan NSW, NT, VIC and regional TAS had the highest usage of MDMA. Methamphetamine was constantly high across all regional and metropolitan sites. In the current study, the NPS ethylone, methoxetamine and alpha were confirmed in metropolitan SA, TAS, WA; regional VIC and regional NT, SA, QLD and WA, respectively, which were some of the regions that had the lowest usage of cocaine and MDMA in the ACIC wastewater

report. It would be very interesting to conduct future screening analysis to see whether these discrepancies hold.

Conclusion

The analysis of municipal wastewater for drugs of abuse has become an important technique which provides complementary information on spatial trends of community drug consumption. A LC-HRMS suspect screening method was successfully used to detect 22 NPS in influent wastewater. This is the first investigation covering the occurrence of NPS on a nationwide scale in Australia, including 50 wastewater treatment plants across all States and Territories. Four classes of NPS were found, emphasising the utility of the applied sample pre-treatment. Of these classes, the cathinones were the most commonly detected. These were present in all States and Territories, in metropolitan as well as regional areas. Five cannabinoids were also observed, but their use was confined to only half of the studied regions. It would be prudent to conduct follow-up studies to investigate any changes in NPS use over time.

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Figure Captions

Figure 1: Confirmation of methoxetamine in a sample from VIC. All confirmation criteria are fulfilled: retention time within 2% of reference standard, protonated molecule and two fragment ions within 2 mDa mass error.

Figure 2: Detection of 5F-APINACA in a sample from QLD. In comparison with the confirmation in Figure 1, only the protonated molecule is seen within 2% of the retention time of the reference standard and mass error within 2 mDa. One fragment ion was seen but it is very close to the level of the noise and thus regarded as such.

Figure 3: Tentative identification of monohydroxylated 5F-APINACA in a sample from QLD. As no reference standard was available for this compound, tentative identification was based on literature findings.(Holm et al., 2015)

Figure 4: All NPS detected and confirmed by site type: regional (red), metropolitan (black) and both (blue) § = compound detected in at least one site; # = compound confirmed in at least one site. No symbol means that the compound was tentatively identified

Table 1: Outline of all NPS detected and confirmed across all sites

Class	Compound	Tentatively identified	Detected	Confirmed
Amphetamine	4-FA		2	0
Amphetamine	MDA		29	15
Cannabinoid	5F-APINACA		2	0
Cannabinoid	5F-APINACA monohydroxylated	1		
Cannabinoid	AM-2201		1	1
Cannabinoid	JWH-018		2	0
Cannabinoid	JWH-073		4	0
Cannabinoid	UR-144		3	1
Cannabinoid	UR-144 *N*-pentanoic acid	1		
Cathinone	4-FMC		2	1
Cathinone	4-MEC		1	0
Cathinone	Alpha PVP		32	11
Cathinone	Butylone		2	0
Cathinone	Ethylone		14	8
Cathinone	Mephedrone		8	0
Cathinone	Methcathinone		41	39
Cathinone	Methylone		13	3
Cathinone	Pentedrone		6	1
Cathinone	Pentylone		3	0
Opioid	U-47700		1	0

Other	Methiopropamine		2	0
Other	Methoxetamine		8	2
Total		2	176	93

Highlights

Most comprehensive screening study of new psychoactive substances

50 sites around Australia covering 60 % of the population were investigated

22 New Psychoactive Substances tentatively identified, detected and/or confirmed

Synthethic Cathinones (methacthinone and alpha PVP) most commonly found

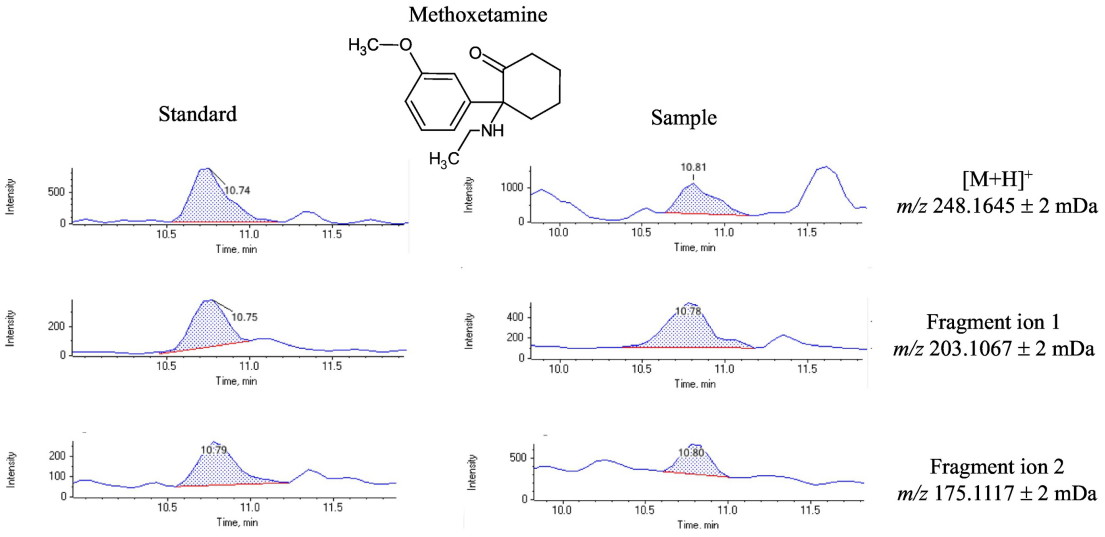

Methoxetamine
H3C—O
O
NH
H3C
Standard
Sample
Intensity
Time, min
10.74
10.81
10.75
10.78
10.79
10.80
[M+H]+
m/z 248.1645 ± 2 mDa
Fragment ion 1
m/z 203.1067 ± 2 mDa
Fragment ion 2
m/z 175.1117 ± 2 mDa

Figure 1

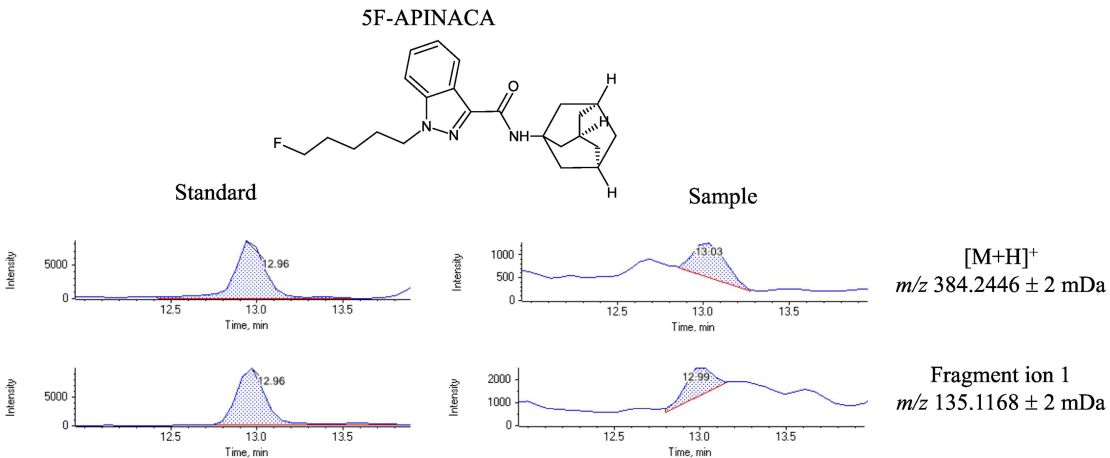
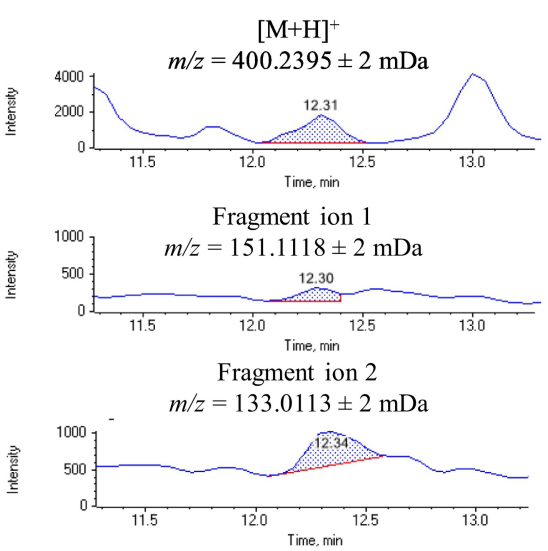
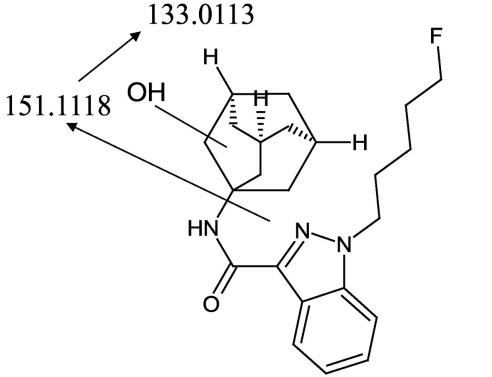

5F-APINACA
F
N
N
O
NH
H
H
H
Standard
Sample
Intensity
5000
0
12.96
12.5
13.0
13.5
Time, min
1000
500
13.03
2000
1000
12.99
[M+H]+
m/z 384.2446 ± 2 mDa
Fragment ion 1
m/z 135.1168 ± 2 mDa

Figure 2





Monohydroxylated 5F-APINACA
m/z = 400.2395

Figure 3

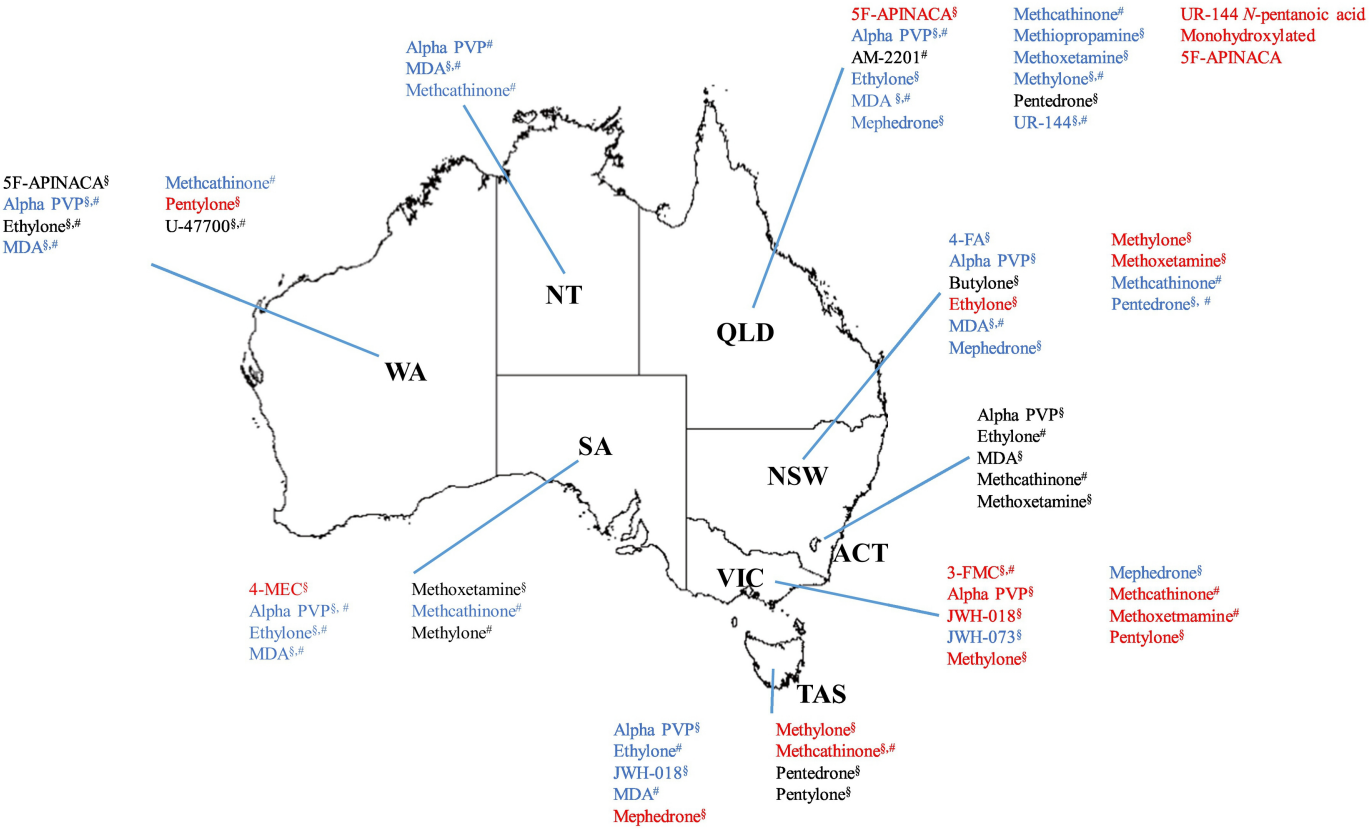

5F-APINACA§
Alpha PVP§,#
AM-2201#
Ethylone§
MDA §,#
Mephedrone§
Methcathinone#
Methiopropamine§
Methoxetamine§
Methylone§,#
Pentedrone§
UR-144§,#
UR-144 N-pentanoic acid
Monohydroxylated
5F-APINACA
Alpha PVP#
MDA§,#
Methcathinone#
5F-APINACA§
Alpha PVP§,#
Ethylone§,#
MDA§,#
Methcathinone#
Pentylone§
U-47700§,#
NT
WA
QLD
SA
NSW
ACT
VIC
TAS
4-FA§
Alpha PVP§
Butylone§
Ethylone§
MDA§,#
Mephedrone§
Methylone§
Methoxetamine§
Methcathinone#
Pentedrone§, #
Alpha PVP§
Ethylone#
MDA§
Methcathinone#
Methoxetamine§
4-MEC§
Alpha PVP§, #
Ethylone§,#
MDA§,#
Methoxetamine§
Methcathinone#
Methylone#
3-FMC§,#
Alpha PVP§
JWH-018§
JWH-073§
Methylone§
Mephedrone§
Methcathinone#
Methoxetmamine#
Pentylone§
Alpha PVP§
Ethylone#
JWH-018§
MDA#
Mephedrone§
Methylone§
Methcathinone§,#
Pentedrone§
Pentylone§

Figure 4